IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ngai et al.

Serial No. 09/597,608

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For: *Normalizing and Amplifying RNA*

Group Art Unit: 1655

Examiner: Taylor, J.

Attorney Docket No. B00-100-1

CERTIFICATE OF TRANSMISSION
I hereby certify that this corr is being transmitted by facsimile to the Comm for Patents at (703) 305-3014 on March 15, 2001.

Signature _____
Richard Aron Osman

RESPONSE

The Commissioner for Patents
Washington, DC 20231

Dear Examiner Taylor:

Thank you for the Office Action dated Feb. 26, 2001.

35USC131

The present Office Action is the second, consecutive non-final examination on the merits of this application. This Action exceeds the Commissioner's authority to subject Applicants to "an examination" (not multiple, piecemeal examinations) under 35USC131. See also, 37CFR1.104(b) which provides that an Examiner's Action "will be complete as to all matters." On this basis, we submit that the Commissioner has exhausted statutory authority to further reject this application, and on this basis alone we request immediate allowance of the application.

35USC102 and 35USC103

For more than twenty-five years, the CCPA and the Federal Circuit have pointedly expressed frustration with Examiners who persist in making unlawful and illogical rejections based on some notion that printed matter limitations may be ignored. Incredibly, this practice continues.

Claims 19 and 20 are in compliance with 35USC102 and 35USC103. The pending art

FIG. 1 illustrates the structure and composition of enamel through a perspective view (i.e., FIG. 1A) and a plan view (i.e., FIG. 1B), respectively, of a section of enamel tissue; and

FIG. 2 illustrates a cross-section of a tooth clarifying the structure and composition of dentin.

EXAMPLE 1

Preparation of Etching Compositions

Aqueous solutions of citric acid at saturation, pH about 1, ortho-phosphoric acid, at a concentration of 37% by weight, pH about 1, and EDTA at a concentration of 25%, pH about 7, are prepared for use in the experiments.

EXAMPLE 2

Preparation of an EDTA-Composition

An aqueous composition is prepared by dissolving about 25% of EDTA in water using NaOH as a pH-controlling agent to give a pH about 7. To the resulting solution sodiumcarboxymethylcellulose is added to form a gel-like viscous composition which is easy to handle in the conditioning procedure and does not flow away from the area to be treated. For obtaining a suitable viscosity a quantity of sodiumcarboxymethylcellulose within the range about 3 to about 5% is suitable.

EXAMPLE 3

Preparation of a Conventional Etching Composition

An aqueous solution of ortho-phosphoric acid having an acid concentration of about 37% is prepared resulting in a pH of the solution of about 1. The resulting solution is made viscous in the same manner as described in Example 2 above.

EXAMPLE 4

Extracted human teeth having dental cavities with exposed dentin and enamel are immersed into the aqueous solutions prepared according to Example 1 above. The teeth are held in the solutions for more than 10 min and are then prepared for examination.

All the solutions remove smear and debris within a short period of time (less than 1 min). Conventional acid etching using citric or phosphoric acid produce an essentially smooth dentin surface with only occasional morphous deposits in the area between the dentinal tubules, but no fibers are visible. Dentinal tubules are clearly visible and appear widened. Etching using EDTA produces a completely different texture with the dentin in-between dentinal tubules consistently displaying a fibrous mesh-work with individual fibers clearly visible and comparable in size to collagenous fibers. Conventional acid etching errodes enamel rods already after 5 to 20 seconds uncovering protruding rod sheaths. EDTA produces similar result only upon exposure for more than 10 min.

The results from the experiments performed show that the collagenous matrix is left intact following EDTA etching, while etching with conventional etching agents, such as citric or phosphoric acid, will dissolve both the mineral and the collagenous matrix of dentin. Etching using EDTA of cavities in preparation for bonding of resin-based fillings are therefore preferred over etching using citric or phosphoric acid. Etching of enamel with EDTA is, however, clinically

impractical due to the need for exposure times in excess of 10 min. Therefore, enamel is preferably etched with a conventional etching acid, such as citric or phosphoric acid, the etching taking less than 20 seconds for obtaining the desired result. Thus, the invention is based on the concept of using different etching compositions for selective use on dentin and enamel, the compositions being based on EDTA and a conventional etching acid, respectively. For practical purposes the etching compositions are preferably in a viscous form to allow full control of application and to avoid accidental etching of dentin with conventional etching acid, such as phosphoric acid. It is to be noted that etching the dentin part of the dental cavity using EDTA need not be restricted to the dentin part only, since the EDTA composition does not deleteriously affect the enamel part of the cavity. However, care should be taken that the conventional etching acid composition is excluded from coming into contact with the dentin part of the dental cavity.

What is claimed is:

1. A kit for use in the conditioning of dental cavities by etching in preparation for bonding restorations to enamel and dentin, comprising the following items:

a) a first container holding an aqueous composition containing EDTA in a concentration which is no less than about 90% of the concentration at saturation of said "acid" has been changed to EDTA;

b) a second container holding an aqueous composition containing an etching acid selected from the group consisting of phosphoric acid and citric acid; and

c) instructions for the use of the kit by etching the dentin part of a dental cavity using said aqueous composition containing EDTA, and by etching the enamel part of said cavity using said aqueous compositions containing said etching acid.

2. A kit according to claim 1, wherein said acid of said second container b) has a pH of about 1.

3. A kit according to claim 2, wherein said acid of said second container b) is phosphoric acid present in a concentration not exceeding about 40% by weight.

4. A kit according to claim 3, wherein the composition of the first container contains, based on the water contents of the composition:

EDTA in an amount of about 22 to 27% by weight;

sodium hydroxide as a pH-controlling agent in an amount resulting in a pH within the range about 6.5 to about 7.5; and

carboxymethyl cellulose (CMC) or a salt thereof as a viscosity-increasing agent in an amount of from about 1% by weight to about 5% by weight.

5. A kit according to claim 1, wherein the composition of the first container a) contains, based on the water contents of the composition:

EDTA in an amount of about 22 to 27% by weight;

sodium hydroxide as a pH-controlling agent in an amount resulting in a pH within the range about 6.5 to about 7.5; and

carboxymethyl cellulose (CMC) or a salt thereof as a viscosity-increasing agent in an amount of from about 1% by weight to about 5% by weight.

6. A kit according to claim 5, wherein the respect to the composition of the first container a):

the amount of EDTA is about 25% by weight;

the pH of the composition is around neutral pH 7; and

the viscosity-increasing agent is sodium carboxymethyl cellulose in an amount of about 3 to 5% by weight.

R3 is selected from the group consisting of Ala, Tyr, and $Tyr(PO_3)_2$;

R4 is selected from the group consisting of Val, Ala, Ile, Norleu, and Leu;

R5 is Phe, Ile, or is absent; and

wherein the active agent is not AII.

5. The method of claim 4 wherein the active agent is selected from the group consisting of SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, and SEQ ID NO:42.

6. A kit for increasing blood flow to ischemic cardiac tissue, comprising:

(a) an amount effective for increasing blood flow to ischemic cardiac tissue of at least one active agent comprising a sequence selected from the general formula

R1-Arg-R2-R3-R4-His-Pro-R5,

wherein

R1 is Asp or is absent;

R2 is selected from the group consisting of Val, Ala, Ile, Pro, Lys, Norleu, and Leu;

R3 is selected from the group consisting of Ala, Tyr, and $Tyr(PO_3)_2$;

R4 is selected from the group consisting of Val, Ala, Ile, Norleu, and Leu;

R5 is Phe, Ile, or is absent;

wherein the active agent is not AII; and

(b) instructions for using the amount effective of active agent for increasing blood flow to ischemic cardiac tissue.

7. The kit of claim 6 wherein the active agent is selected from the group consisting of SEQ ID NO:4, SEQ ID NO:19, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42.

8. The kit of claim 6, further comprising a means for delivery of the active agent.

* * * * *

FDNC Blue #1. The essential characteristic of the dye is that it be soluble in the liquid being collected.

The individual collection apparatuses of the invention can be stored in a fluid collection kit comprising multiple tubes of the two types described above and multiple caps. The kit will normally comprise a container adapted to hold the tubes and caps in a readily accessible manner (typical of the type used in a test tube rack in which the individual tubes are inserted into holes in a rack-like device, typically made of cardboard in a commercial collection kit). The individual tubes can have built-in labels for ease of use (for example, containing spaces for patient name and date and time of collection), and written instructions adapted for the particular type of sample can be included in the box that holds the individual tubes.

The instant invention further provides kits for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising a fluid collection device, such as those described above, and instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman by using the methods of the invention described above.

It is to be understood that the invention is not to be limited to the exact details of operation, or to the exact compounds, compositions, methods, procedures or embodiments shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope of the appended claims.

We claim:

1. A method for monitoring the effectiveness of hormone replacement therapy in a perimenopausal woman, comprising

a. obtaining a body fluid sample from the perimenopausal woman;

b. testing for hormonal levels in the body fluid of the perimenopausal woman at intervals of between about 7 days to about 90 days, wherein testing for hormonal levels comprises testing for levels of at least one of progesterone, testosterone, estradiol, follicle stimulating hormone, and estriol; and

c. adjusting levels of replacement hormone administered to the woman based on the test results.

2. A method for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising:

a. obtaining a body fluid sample from the perimenopausal woman;

b. testing for hormonal levels in the body fluid of the perimenopausal woman at intervals of between about 30 days to about 180 days, wherein testing for hormonal levels comprises testing for levels of at least one of progesterone, testosterone, estradiol, follicle stimulating hormone, and estriol; and

c. adjusting levels of replacement hormone administered to the woman based on the test results.

3. The method of claim 1 wherein the body fluid is saliva.

4. The method of claim 2 wherein the body fluid is saliva.

5. A kit for monitoring the effectiveness of hormone replacement therapy in a perimenopausal woman, comprising:

a. a fluid collection device;

b. instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman according to the method of claim 1.

6. A kit for monitoring the effectiveness of hormone replacement therapy in a early menopausal or late menopausal woman, comprising:

a. a fluid collection device;

b. instructions for using the fluid collection device for monitoring the effectiveness of hormone replacement therapy in a menopausal woman according to the method of claim 2.

* * * * *

wherein the snare cords are arranged about the pins in the loose knot pattern.

4. A knot tying device as in claim 3, wherein the pins are arranged in a pattern having a cluster of four pins at one end and a line of at least three pins extending from the cluster.

5. A knot tying device as in claim 1, wherein the capture end of each snare cord comprises a loop for receiving a suture end therethrough.

6. A knot tying device as in claim 1, wherein the pull end of each snare cord comprises a handle to permit manual grasping thereof.

7. A knot tying device as in claim 1, wherein the knot is selected from the group consisting of a square knot, a sliding surgeon's knot, a square knot, a cinch knot, a capstan knot, and a reef knot.

8. A knot tying device comprising:

a handle;

a knotting guide attached to the handle, said knotting guide including a plurality of guide pins arranged in a pre-defined pattern and a cover which can be selectively opened and closed over the pins;

a first snare cord having a capture end and a pull end arranged over the pins in a partial knot pattern; and

a second snare cord having a capture end and a pull end arranged over the pins in a knot pattern complementary to the partial knot pattern;

whereby a knot may be formed in first and second suture ends by capture of the first suture end with the capture end of the first snare cord, capture of the second suture end with the capture end of the second snare cord, pulling of the first snare cord through the pins so that the first suture end assures the partial knot pattern, pulling of the second snare cord through the pins to assure the complementary knot pattern, opening of the cover, and removal of the knot in the suture ends from the knotting guide.

9. A knot tying device as in claim 8, wherein the pins are arranged in a pattern having a cluster of four pins at one end and a line of at least three pins extending from the cluster.

10. A knot tying device as in claim 8, wherein the capture end of each snare cord comprises a loop for receiving a suture end therethrough.

11. A knot tying device as in claim 8, wherein the pull end of each snare cord comprises a handle to permit manual grasping thereof.

12. A knot tying device as in claim 8, wherein the knot is selected from the group consisting of a square knot, a sliding surgeon's knot, a square knot, a cinch knot, a capstan knot, and a reef knot.

13. A kit comprising a knot tying device as in claim 1, and a package, wherein the device is sterile and contained in the package.

14. A kit comprising:

a knot tying device including:

a first snare cord having a capture end and a pull end;

a second snare cord having a capture end and a pull end; and

instructions for use setting forth the following steps:

capturing a first suture end with the capture end of the first snare cord;

capturing a second suture end with the capture end of the second snare end;

pulling the first snare cord through a path which defines a partial knot pattern; and

pulling the second snare cord through a path which defines a complementary knot pattern;

whereby the suture ends are formed into a knot.

15. A method for tying a first suture end and a second suture end into a knot, said method comprising:

capturing the first suture end with a first snare cord;

capturing the second suture end with a second snare cord;

pulling the first snare cord through a path which defines a partial knot pattern; and

pulling the second snare cord through a path which defines a knot pattern complementary to the partial knot pattern;

whereby the suture ends are formed into a complete knot.

16. A method as in claim 15, wherein the first suture end and the second suture end comprise opposite ends of a single length of suture.

17. A method as in claim 16, wherein the single length of suture is anchored in tissue.

18. A method as in claim 16, wherein the single length of suture passes through penetrations on the periphery of a blood vessel puncture.

19. A method as in claim 16, wherein the single length of suture passes between blood vessels in an anastomotic attachment.

20. A method as in claim 16, wherein the single length of suture passes between a prosthetic device and tissue.

21. A method as in claim 15, wherein the capturing steps both comprise placing the suture end in a loop formed in the end of the snare cord.

22. A method as in claim 21, wherein the pulling steps both comprise drawing the snare cord through a knotting guide which holds the first snare cord and the second snare cord in a loose knot pattern prior to the pulling steps.

23. A method as in claim 22, wherein the two pulling steps are performed sequentially.

24. A method as in claim 22, wherein the two pulling steps are performed simultaneously.

25. A method as in claim 15, further comprising advancing the complete knot to engage a tissue surface.

26. A method as in claim 15, wherein the complete knot comprises a sliding knot having a rail end and a cinching end, wherein the knot is advanced by holding the rail end and pushing the knot.

27. A method as in claim 15, wherein the complete knot comprises a square knot.

28. A knotting device comprising:

a knotting guide which imparts a loose knot pattern in a first suture end and a second suture end; and

a shaft removably secured to the knotting guide, the shaft defining a distal passage for receiving one of the first and second suture ends, wherein said shaft is positioned adjacent to the loose knot after the knot has been formed and the knotting guide has been removed, so that the shaft can be advanced over the one of the first and second suture ends advance the knot along said one of the first and second suture ends.

29. A knotting device as in claim 28, wherein the knotting guide comprises:

a first snare cord having a capture end and a pull end;

a second snare cord having a capture end and a pull end; and

a knotting guide which holds the first snare cord and the second snare cord in a loose knot pattern so that a knot may be formed in a first suture end and a second suture

in treating neurodegenerative pathologies not significantly affecting memory or learning. Preferred compounds and unit doses include those described herein above.

We claim:

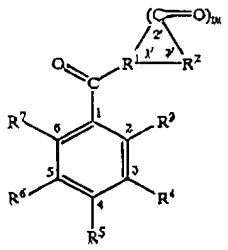
1. A method for treating schizophrenia in a subject, said method comprising administering an effective amount of a composition that comprises a first compound that enhances the stimulation of α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid ("AMPA") receptors in said subject and a second antipsychotic compound.

2. The method of claim 1 wherein the composition is administered orally.

3. The method of claim 1 wherein the composition is administered by injection.

4. A kit, comprising a container containing the composition of claim 1 and instructions for using the composition for treating schizophrenia in a subject.

5. A method in accordance with claim 1 wherein said first compound has the following formula, with ring vertices numbered as shown:



in which:

R^1 is a member selected from the group consisting of N and CH;

m is 0 or 1;

R^2 is a member selected from the group consisting of $(CR^8_2)_{n-m}$ and $C_{n-m}R^8_{2(n-m)-2}$, in which n is 4, 5, 6, or 7, the R^8's in any single compound being the same or different, each R^8 being a member selected from the group consisting of H and C_1–C_6 alkyl, or one R^8 being combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2, CH_2—CH_2, CH═CH, O, NH, N(C_1–C_6 alkyl), N═CH, N═C(C_1–C_6 alkyl), C(O), O—C(O), C(O)—O, CH(OH), NH—C(O), and N(C_1–C_6 alkyl)—C(O);

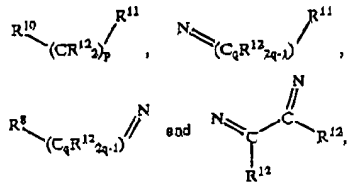
R^3, when not combined with any R^8, is a member selected from the group consisting of H, C_1–C_6 alkyl, and C_1–C_6 alkoxy;

R^4 is either combined with R^5 or is a member selected from the group consisting of H, OH, and C_1–C_6 alkoxy;

R^5 is either combined with R^4 or is a member selected from the group consisting of H, OH, C_1–C_6 alkoxy, amino, mono(C_1–C_6 alkyl)amino, di(C_1–C_6 alkyl) amino, and CH_2OR^9, in which R^9 is a member selected from the group consisting of H, C_1–C_6 alkyl, an aromatic carbocyclic moiety, an aromatic heterocyclic moiety, an aromatic carbocyclic alkyl moiety, an aromatic heterocyclic alkyl moiety, and any such moiety substituted with one or more members selected from the group consisting of C_1–C_3 alkyl, C_1–C_3 alkoxy, hydroxy, halo, amino, alkylamino, dialkylamino, and methylenedioxy;

R^6 is either H or CH_2OR^9;

R^4 and R^5 when combined form a member selected from the group consisting of



in which

R^{10} is a member selected from the group consisting of O, NH and N(C_1–C_6 alkyl);

R^{11} is a member selected from the group consisting of O, NH and N(C_1–C_6 alkyl);

R^{12} is a member selected from the group consisting of H and C_1–C_6 alkyl, and when two or more R^{12}'s are present in a single compound, such R^{12}'s are the same or different;

p is 1, 2, or 3; and

q is 1 or 2; and

R^7, when not combined with any R^8, is a member selected from the group consisting of H, C_1–C_6 alkyl, and C_1–C_6 alkoxy.

6. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_{n-m}$ and $C_{n-m}HR^8_{2(n-m)-3}$, and R^3 is a member selected from the group consisting of H, C_1–C_6 alkyl, and C_1–C_6 alkoxy.

7. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_{n-m}$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2, CH_2—CH_2, CH═CH, O, NH, N(C_1–C_6 alkyl), N═CH, N═C(C_1–C_6 alkyl), C(O), O—C(O), C(O)—O, CH(OH), NH—C(O), and N(C_1–C_6 alkyl)—C(O).

8. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_{n-m}$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single bond linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices or a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2, CH_2—CH_2, CH—CH, O, NH, C(O), and CH(OH).

9. A method in accordance with claim 5 in which R^2 is a member selected from the group consisting of $(CHR^8)_{n-m}$ and $C_{n-m}HR^8_{2(n-m)-3}$, and one R^8 is combined with either R^3 or R^7 to form a single divalent linking moiety linking the no. 3' ring vertex to either the no. 2 or the no. 6 ring vertices, the linking moiety being a member selected from the group consisting of CH_2, O, NH, C(O), and CH(OH).

10. A method in accordance with claim 5 in which m is zero, R^2 is a member selected from the group consisting of CHR^8—CH_2—CH_2—CH_2 and CHR^8—CH_2—CH_2—CH_2—CH_2, in which R^8 is combined with R^7 to form a single divalent linking moiety linking the 2 and 3' ring vertices, the linking moiety being a member selected from the group consisting of CH_2, O, NH, C(O), and CH(OH).

regulatory pathway or a gene product, is an expensive multistep process, with biochemical or molecular cloning approaches. Applying the immunohistochemical procedure as described, equipped with a broad spectrum antibody to a kinetic regulatory protein, a straight forward identification of defects in its translocation are identified first. Next, a comparison of structural components of a normal translocator versus defective non-translocator will provide a clue regarding those transcription factors which are essential to induce intracellular translocation of a nuclear binding component.

What I claim is:

1. An immunohistochemical method to detect normal and abnormal intracellular movement of a kinetic cellular component from one cellular compartment to another, comprising the steps of:

a) pre-coating the glass slides with a solution containing a ligand specific to the kinetic component to be examined, a control ligand or no ligand;

wherein a specific ligand is an agent or a compound which upon incubation with the said kinetic component effects its intracellular movement from one compartment to another;

wherein a control ligand does not upon incubation effect an intracellular movement of the said kinetic component;

b) imprinting tumor tissue, thaw mounting cryosections of normal or tumor tissue or smearing core-needle biopsies or cell suspensions on uncoated or said pre-coated glass slides;

c) incubating the slides in moist chamber to allow for ligand binding;

d) fixing the incubated tissue;

e) detecting the kinetic component by binding an antibody to the said kinetic component;

f) determining the normal or abnormal movement of the said kinetic component by comparing its cellular location when incubated with or without the specific ligand.

2. The immunohistochemical method of claim 1 whereby breast tumors are classified based upon the presence in tumor cells of normal or defective estrogen receptors,

wherein, estrogen receptor is the kinetic component;

wherein, normal estrogen receptor upon incubation with estrogen receptor-specific ligand moves from the cytoplasm to the nuclear compartment of the cell and the tumor with said normal receptors is classified as Tr+L;

wherein, defective estrogen receptor upon incubation with estrogen receptor-specific ligand fails to move from cytoplasm to the nuclear compartment of the cell and the tumor with said defective estroegen receptors are classified as Tr(−);

wherein, defective estrogen receptors upon incubation with no ligand or with control ligand moves from the cytoplasm to the nuclear compartment of the cell and the tumor with said defective estrogen receptors is classified as Tr+NL;

wherein, the detection of normal and defective estrogen receptor is accomplished by:

a) imprinting tumor tissue, thaw mounting cryosections of tumor or normal tissue, smearing core-needle biopsy or cell suspension on estrogen receptor-specific ligand coated or control ligand coated slides;

b) incubating the slides in moist chamber to allow ligand binding;

c) fixing the incubated tissue;

d) detecting the estrogen receptor by binding an antibody to estrogen receptor;

e) determining the percentage of cells with normal or defective estrogen receptors and classifying as normal or Tr+L, defective or Tr(−), or as defective or TR+NL variety.

3. The immunohistochemical method of claim 2 whereby breast tumor patients are selected for hormonal or other non-hormonal modes of treatments;

wherein patients with tumors of Tr+L variety containing normal estrogen receptors are predicted to be responders to hormonal therapy and thereby selected for hormonal treatments;

wherein patients with tumors of Tr(−) and /or Tr+NL varieties containing defective estrogen receptors are predicted to be non-responsive to hormonal modes of therapy and thereby selected for non-hormonal modes of therapies.

4. An immunohistochemical method of screening for the ligand specific for a kinetic cellular component

wherein a specific ligand is an agent or a compound that upon incubation with the kinetic cellular component effects its intracellular movement from one compartment to another;

wherein estrogen receptor is a kinetic cellular component and specific ligand upon incubation effects the movement of estrogen receptor from the cytoplasm to the nuclear compartment of the cell ligand screening comprise the steps of:

a) precoating glass slides with a solution of ligand known to be specific to estrogen receptor, the solution to be screened for the presence of a specific ligand or a solution of control ligand;

b) imprinting tumor tissue with normal estrogen receptor, thaw-mounting cryosections of tumor or normal tissue with estrogen receptor, smearing cell suspension from estrogen receptor containing tissue onto said pre-coated slides;

c) incubating the slides in a moist chamber to allow ligand binding;

d) fixing the incubated tissue;

e) detecting the estrogen receptor by binding an antibody specific to estrogen receptor;

f) determining the cellular location of estrogen receptor in the presence vs absence of the solution being screened for ligand.

5. The immunohistochemical ligand screening method of claim 4, whereby screening for an inhibitor-ligand is accomplished,

wherein an inhibitor-ligand is an agent or a compound which when combined with an estrogen receptor specific ligand, prevents the estrogen receptor from moving from the cytoplasm to the nuclear compartment of the cell;

and the method of screening further comprising the steps of:

a) precoating glass slides with a solution containing estrogen receptor specific ligand, a solution of control ligand or a solution of specific ligand mixed with the solution to be screened for an inhibitor-ligand;

b) following steps b-e specified for claim 4;

c) determining the location of estrogen receptor in the presence of specific ligand and in the mixture containing both the specific ligand and the ligand being examined.

6. An immunohistochemical method for screening for monoclonal anti-estrogen receptor antibodies with exclusive specificity for either normal Tr+L, defective Tr(−) or defective Tr+NL estrogen receptors, wherein to normal/Tr+L receptors are those which need binding to the specific ligand to move from cytoplasm to the nuclear compartment of the cell;

wherein defective Tr(−) estrogen receptors are those which do not move from cytoplasm to the nuclear compartment even upon binding to estrogen receptor specific ligand;

wherein defective Tr+NL estrogen receptors are those which will move from cytoplasm to nuclear compartment of the cell even in the absence of estrogen receptor specific ligand;

wherein the antibody screening comprises the steps of:

a) imprinting on glass slide, or thaw mounting cryosections of normal tissue or tumor tissue with Tr+L estrogen receptors, Tr(−) estrogen

b) air drying the tissue followed by fixing the tissue on the slide;

c) contacting the fixed tissue with solution containing monoclonal antiestrogen receptor antibody;

d) determining the antibody binding to Tr+L, Tr(−) or Tr+NL estrogen receptors or to all three by immunohistochemical staining techniques.

7. A kit intended for the purpose as described in claim 1 and comprising any of the following either singly or in combination

a) Ligand-coated glass slides

b) saline or buffer-coated glass slides

c) tissue or tumor imprinted glass slides

d) glass slides smeared with cell suspensions

e) instructions with procedural details described in claim 1.

* * * * *

TABLE 5-continued

Spleen weight
(† = animal died untimely)

Treatment group	Spleen weight (g) after 4 weeks treatment	Spleen weight (g) after 2 weeks with no treatment
	0.492	0.261
	0.483	0.153
	0.486	0.273
Median value	0.491	0.224
CY + G-CSF	0.263	0.177
	0.218	0.228
	0.225	0.208
	0.324	0.218
	0.254	0.232
Median value	0.257	0.213

TABLE 6

Leukocyte number (WBC)
(† = animal died untimely)

Treatment group	Animal number	WBC (× $10^6/\mu l$) Time (weeks) 0	1	2	3	4	6
Control	1	5.6	5.2	9.4	3.3	6.6	—
	2	10	6.3	6.9	†	†	†
	3	7	4.8	7	3.4	6.8	—
	4	7.6	7.6	7.7	3.9	7.7	
	5	9.1	6	11	3.5	6.3	—
	6	8.4	5.1	9.5	6.2	7.5	4.5
	7	2.5	2.3	5.3	5	5.7	3
	8	9.5	7.3	12.2	8.5	13.7	7.8
	9	6.3	4.3	8.8	4.2	10.1	4.5
	10	6	5.9	8.5	5.9	8	6.4
Median value		7.20	5.48	8.63	4.88	8.04	5.24
CY alone	1	4.8	2.9	5	7.6	4	—
	2	9	7.1	5.3	2.7	2.8	—
	3	8.6	5.9	7.3	5.2	7	—
	4	6.3	4.8	6.3	6.1	4.8	—
	5	5.3	4.5	4.9	8.6	2.9	—
	6	6.4	5	5.1	6.7	4.1	3.6
	7	6.8	4.7	4.9	5	3.6	3.2
	8	5.6	5.7	4.8	4.1	3.7	4.6
	9	5.8	4.7	5.9	4.1	7.1	5.9
	10	9.4	6	7.1	4	2.8	5.9
Median value		6.80	5.13	5.66	5.41	4.28	4.64
G-CSF + CY	1	6.1	11.7	27.7	33.4	39.4	—
	2	6.8	12.4	46.5	43.7	30.5	—
	3	5.6	11.2	30.3	58.78	40.6	—
	4	4.3	11.7	35.3	51.4	40.7	—
	5	6.2	22.4	62.2	55.4	66.7	—
	6	6.2	12.6	49.2	84.2	85.4	—
	7	7.1	16.8	84.8	105.4	102.3	—
	8	8	18.6	82	117.6	70.1	—
	9	3	5.4	42.7	15.8	33.3	2.2
	10	4.6	18.2	62.8	69	33.2	3.9
	11	5.8	10.9	29.1	40	33.5	2.7
	12	5	12.7	43.8	51.9	28.1	6.4
	13	5.8	14.6	28.8	33.4	20	4.6
	14	6.2	10.2	50	36.8	45.4	3.6

TABLE 6-continued

Leukocyte number (WBC)
(† = animal died untimely)

Treatment group	Animal number	WBC (× $10^6/\mu l$) Time (weeks) 0	1	2	3	4	6
	15	8.6	20.2	30	36.2	25.3	5.2
	16	5.9	12.8	26.8	45.5	54.8	4.9
Median value		6.00	14.04	48.08	55.16	44.60	4.19
CY + G-CSF	1	6.7	6.1	6.9	9.5	12.68	—
	2	9.1	7.1	11.08	11.6	3.78	—
	3	9.1	11.1	4.5	9.6	5.9	—
	4	6.9	5.2	10.4	16.6	5	—
	5	6.3	3.9	7	#	11.6	—
	6	9.6	8.3	5	6.9	4.1	4.6
	7	6.6	6.3	8.6	7.1	7.5	4.2
	8	6.1	4.4	5.4	3.8	3.18	2.4
	9	10.3	7.6	4.8	4.2	6.2	3.7
	10	8.2	6.5	5.4	6.2	4.3	3.2
Median value		7.89	6.65	6.91	8.39	6.42	3.62

What is claimed is:

1. A method of treating a disease requiring peripheral stem cell transplantation in a patient in need of such treatment, comprising administering to the patient a hematopoietic stem cell mobilizing-effective amount of G-CSF; and thereafter administering to the patient a disease treating-effective amount of at least one chemotherapeutic agent.

2. The method of claim 1, wherein the disease is a tumor disease.

3. The method of claim 1, wherein the G-CSF is recombinant G-CSF.

4. The method of claim 1, wherein the at least one chemotherapeutic agent opens the endothelial barrier of the patient to render the endothelial barrier permeable for stem cells.

5. The method of claim 1, wherein the at least one chemotherapeutic agent is cyclophosphamide.

6. The method of claim 1, wherein the G-CSF is administered once per day over 2–3 consecutive days, and the chemotherapeutic agent is administered immediately after the final administration of G-CSF, or on a fourth consecutive day.

7. A pharmaceutical kit, comprising

a first component comprising G-CSF;

a second component comprising at least one chemotherapeutic agent; and

a third component comprising instructions for the administration of the G-CSF prior to the onset of administration of the at least one chemotherapeutic agent.

* * * * *

has been snapped into the locked position, thereby securing the curling ribbon **1200** to the strand of hair **70**.

As shown in FIG. **14**, a lower portion of the right hand side **1212** of the curling ribbon **1200** is first folded over the strand **70**, and then the left hand side **1214** is folded over the right hand side **1212** in the direction of arrow **1215** so as to envelop the strand **70** inside the curling ribbon **1200**. The curling ribbon **1200** is then wound around a curling mandrel, and the mandrel is withdrawn in the same way as illustrated in FIGS. 7 through **9** with respect to curling ribbon **400**. When the ribbon **1200** is unwrapped from the curled hair, you use your fingers to unsnap the locking comb **1280** to release the hair strand **70**, which unsecures the curling ribbon **1200** from the hair.

As shown in FIG. **12**, the locking comb **1280** may be attached to the curling ribbon **1200** by base stiffener **1240** of wire extending through holes **1284**. Alternatively, the locking comb **1280** may be attached to the curling ribbon **1200** by sewing it through holes **1284** to the fabric **1210**, and in that event the base stiffener **1240** may optionally be omitted. In either event the end stiffener **1250** may optionally be omitted from the curling ribbon when a locking comb **1280** is used.

Modifications to the embodiments described above may be made without departing from the basic spirit of the present invention. Accordingly, it will be appreciated by those skilled in the art that, within the scope of the appended claims, the invention may be practiced other than has been specifically described herein.

I claim:

1. A method for curling hair comprising the following steps:

Isolating a strand of hair that the subject desires to be curled;

Providing a curling ribbon comprising a ribbon of fabric having a plurality of bendable stiffening elements extending longitudinally thereof;

Placing the curling ribbon adjacent the strand of hair and wrapping it around the strand to envelop substantially the entire hair strand;

Securing the curling ribbon to hair at or near the base of the strand;

Providing a curling mandrel of generally cylindrical shape having a diameter the size of the desired curls, and placing it adjacent the base of the hair strand to which the curling ribbon has been secured;

Wrapping the curling ribbon helically around the curling mandrel until substantially the entire strand of hair is coiled around the curling mandrel within the curling ribbon;

Withdrawing the curling mandrel axially from within the coil without uncoiling; the strand;

Applying a curling agent to the strand; and

Permitting the strand to remain coiled within the curling ribbon until the curling agent has curled the strand of hair.

2. The method of claim **1**, wherein the curling agent is a setting composition.

3. The method of claim **2**, wherein the setting composition is applied to the strand before the curling ribbon is wrapped around the strand.

4. The method of claim **1**, wherein the curling agent is a permanent waving composition.

5. The method of claim **4**, wherein the setting composition is applied to the strand after the curling mandrel has been withdrawn.

6. The method of claim **1**, wherein the curling ribbon is secured to the strand of hair by a spring-loaded clip.

7. The method of claim **1**, wherein the curling ribbon further comprises a comb at its base end and the curling ribbon is secured to the strand of hair by said comb.

8. The method of claim **7**, wherein said comb is a locking comb of flexible metal.

9. The method of claim **1**, further comprising the step of:

Removing the curling ribbon from around the strand and unsecuring it from the strand, so that the curled hair strand may be combed out.

10. The method of claim **1**, further comprising the steps of:

Isolating additional strands of hair on the subject's head;

Providing an additional curling ribbon for applying to each of said additional strands of hair;

Placing one the additional curling ribbons adjacent each of the additional strands of hair and wrapping it around the strand to envelop substantially the entire hair strand;

Securing the additional curling ribbon to hair at or near the base of the strand;

Placing said curling mandrel adjacent the base of each of the additional hair strands to which one of the additional curling ribbons has been secured;

Wrapping the curling ribbon helically around the curling mandrel until substantially the entire strand of hair is coiled around the curling mandrel within the curling ribbon;

Withdrawing the curling mandrel axially from within the coil without uncoiling; the strand;

Applying a curling agent to the strand; and

Permitting each of the additional strands of hair to remain coiled within each of the additional curling ribbons until the curling agent has curled the strands of hair.

11. A curling ribbon adapted for use in the method of claim **1**, comprising

A ribbon of fabric having a base end and a distal end;

A pair of edge stiffeners extending longitudinally along each edge; and

A longitudinal stiffener extending parallel to said edge stiffeners between them.

12. The curling ribbon of claim **11**, further comprising a comb secured to the base end of said ribbon of fabric.

13. The curling ribbon of claim **11**, further comprising a base stiffener.

14. The curling ribbon of claim **11**, further comprising a comb secured to the base stiffener.

15. The curling ribbon of claim **11**, further comprising an end stiffener.

16. The curling ribbon of claim **11**, wherein said ribbon of fabric is between about 5 and 7 centimeters wide.

17. The curling ribbon of claim **11**, wherein said ribbon of fabric is between about 40 and 75 centimeters long.

18. The curling ribbon of claim **11**, wherein said ribbon of fabric is brightly colored.

19. The curling ribbon of claim **11**, wherein said ribbon of fabric bears a decorative design.

20. A kit for use in performing the method of claim **1**, comprising at least 20 curling ribbons, a first curling mandrel, and instructions.

21. The kit of claim **20**, further comprising at least one additional curling mandrel having a different diameter from said first curling mandrel.

22. The kit of claim **20**, further comprising a curling agent.

* * * * *

TABLE 1-continued

Monkey	Fe D 0 μg/ml	Fe D 7 μg/ml	TransFe sat % Day 0	TransFe sat % Day 7	Toxicity Liver & Kidney
N11	135	1	33	−2	L+/−/K−
738PR		1		−4	L+/K+
Mean	109 ± 18	2 ± 2	34 ± 4	−1.2 ± 2	
$p < 0.001$	$p < 0.0001$				

Pathology
the liver- (L+) swelling of hepatocytes, (L++) degeneration, (L+++) lipidosis (#73, #105), regeneration (885N)
the kidneys- (K+) multifocal casts, (K++) glomerulonophritis (#73, #105), hemosiderin deposits (K−) no toxicity
No brain changes (substantia nigra, cerebellum- Purkinje cells)

The results of this study shows that a specific chelator of ferrous iron can prevent vasospasm in a primate model of SAH. In this primate model of SAH-related vasospasm, the reliability of producing spasm in untreated animals is 98% [Pluta et al., J. Neurosurg. 77:740–748 (1992)]. Six animals receiving 70 mg/kg/day of dipyridyl had no arteriographic vasospasm. The serum iron was markedly reduced in the drug-treated animals on day 7, as compared to day 0, confirming chelation of the plasma iron in the treated animals. The percent saturation for the endogenous chelator transferrin was decreased in treated animals, suggesting that dipyridyl strongly chelates iron. Furthermore, toxicity at the effective dose is mild.

What is claimed:

1. A method of preventing or treating cerebral vasospasm comprising:

internally administering to a human who has suffered subarachnoid hemorrhage an effective amount of an iron chelator which preferentially chelates ferrous iron over ferric iron and which competes with nitric oxide binding to ferrous iron, thereby interfering with the nitric oxide sink effect,

wherein said iron chelator is selected from the group consisting of 2,22'-dipyridyl and 1,10-phenanthroline.

2. The method of claim 1, wherein said iron chelator is internally administered through a route selected from the group consisting of a continuous IV, intravenous bolus, and oral.

3. The method of claim 1, wherein said iron chelator is internally administered to the human within 7 days of the subarachnoid hemorrhage.

4. The method of preventing or treating vasospasm of claim 1, wherein the said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

5. The method of claim 2, wherein said route is intravenous bolus.

6. The method for the prevention or treatment of vasospasm of claim 1, wherein said iron chelator is 2,2' dipyridyl.

7. The method of claim 6, wherein said iron chelator is internally administered at a dose of 20–100 mg/kg/day.

8. A kit or labeled container for the prevention or treatment of cerebral vasospasm in a human who has suffered subarachnoid hemorrhage, comprising:

a container;

an effective amount of an iron chelator in said container which preferentially chelates ferrous iron over ferric iron and competes with nitric oxide binding to said ferrous iron thereby interfering with the nitric oxide sink effect; and

instructions either secured to said container or associated with said kit which give directions on how to use said iron chelator for prevention or treatment of cerebral vasospasm in said human who has suffered subarachnoid hemorrhage, wherein said directions provide for internally administering said effective amount of said iron chelator to said human,

wherein said iron chelator is selected from the group consisting of 2,2'-dipyridyl and 1,10-phenanthroline.

9. The kit or labeled container for the prevention or treatment of vasospasm of claim 8, wherein said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

10. The kit or labeled container for the prevention or treatment of vasospasm of claim 8, wherein said iron chelator is 2,2' dipyridyl.

11. A pharmaceutical composition for the prevention or treatment of cerebral vasospasm comprising:

an effective amount of an iron chelator which preferentially chelates ferrous iron over ferric iron and competes with nitric oxide binding to said ferrous iron thereby interfering with the nitric oxide sink effect and wherein said chelator is effective for preventing or treating cerebral vasospasm when administered internally to said human who has suffered subarachnoid hemorrhage; and

a pharmaceutically acceptable carrier,

wherein said iron chelator is selected from the group consisting of 2,2'-dipyridyl and 1,10-phenanthroline.

12. The pharmaceutical composition for the prevention or treatment of vasospasm of claim 11, wherein said iron chelator is 2,2' dipyridyl.

13. The pharmaceutical composition for the prevention or treatment of vasospasm of claim 11, wherein said iron chelator further penetrates the blood-brain barrier and acts intracellularly.

14. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in a concentration to allow the administration of an amount of 20–100 mg/kg/day.

15. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in concentration 0.5–50 mg/ml.

16. The pharmaceutical composition of claim 12, wherein said ferrous iron chelator is present in concentration 5–25 mg/ml.

* * * * *

EXAMPLE 5

In this experiment, milrinone was evaluated for its capability to induce erection in the anesthetized male cat. Adult male cats (3.5 to 4.7 kg) were initially sedated with ketamine and then anesthetized and maintained with supplemental doses of pentobarbital administered through a polyethylene catheter inserted into the left external jugular vein. After exposure of the pubic area, a 25-gauge needle was placed into the left corpus cavernosum for measurement of intracavernous pressure. Milrinone (Sigma Chemical, St. Louis, Mo.) was dissolved in 20% dimethylsulfoxide (DMSO) and diluted with 0.9% NaCl. All drug solutions were stored in a freezer in amber bottles; working solutions were prepared on a frequent basis and kept on crushed ice. Vehicle and various doses of milrinone (3, 10, 30 and 100 μg) were administered by direct injection into the right corpus cavernosum using a 30-gauge needle. Doses of zaprinast (3, 10, 30 and 100 μg) were also given for purposes of comparison (see Table). All injections were made using a total volume of 200 μl. At the end of the experiment, each animal received a control drug solution containing 1.65 mg papaverine, 25 μg phentolamine, and 0.5 μg of prostaglandin E_1 to establish maximal response in each subject.

Intracavernosal injections of milrinone and zaprinast caused dose dependent increases in cavernosal pressure and penile length. The maximal increase in cavernosal pressure in response to milrinone was an approximate five-fold increase from a baseline value of 20±2 mmHg (zaprinast was similar). The 100 μg dose of milrinone produced 81±7% of the erectile response elicited by the standard control combination. The erectile response resulting from the 100 μg dose of milrinone lasted 20±6 minutes, longer than the response to a 100 μg dose of zaprinast (duration 14±1 minutes). These results are illustrated in FIGS. 2 and 3, bar graphs showing measured intracavernosal pressure (FIG. 2) and penile length (FIG. 3) following intracavernosal injections of milrinone (3 to 30 μg). (Data in the figures and in the Table are shown as mean ± standard error of the mean for n experiments per dose; "Triple" denotes the response to the control drug solution.)

The duration of the erectile response increased in a dose-dependent manner with increasing doses of milrinone, up to a maximum of 20±6 minutes at 100 μg, the highest dose tested. Intracavernosal injection of milrinone did not produce significant decreases in systemic arterial pressure, except for a small effect a the highest (100 μg) dose, causing a decrease of 8±3 mm Hg. This was much smaller than the 34±8 mm Hg decrease in systemic arterial pressure caused by the standard control combination. These results are presented in Table 1.

TABLE 1

Duration of Erectile Response and Change in Systemic Arterial Pressure (ΔSAP)

Drug	Dose (μg)	Duration (Minutes)	ΔSAP (mm Hg)
Milrinone	3	7 ± 2	0 ± 0
Milrinone	10	9 ± 1	0 ± 0
Milrinone	30	14 ± 3	−4 ± 3
Milrinone	100	20 ± 6	−8 ± 3
Triple		38 ± 10**	−34 ± 8
Zaprinast	3	7 ± 5	−1 ± 1
Zaprinast	10	10 ± 3	−1 ± 1
Zaprinast	30	12 ± 3	−1 ± 3

TABLE 1-continued

Duration of Erectile Response and Change in Systemic Arterial Pressure (ΔSAP)

Drug	Dose (μg)	Duration (Minutes)	ΔSAP (mm Hg)
Zaprinast	100	14 ± 1	−2 ± 2
Triple		33 ± 7**	−54 ± 6**

FIG. 4 compares the dose response curves obtained from experiments in cats treated with milrinone with the dose response curves obtained from experiments in cats treated with zaprinast (data shown as mean ± standard error of the mean; "n" is the number of experiments for each dose). When compared on a molar basis, the dose-response curves for milrinone and zaprinast were similar, indicating that the two PDE inhibitors had similar activity when administered intracavernosally. These results suggest that a selective Type III phosphodiesterase inhibitor, when administered locally, can induce significant increases in erectile response in a mammalian male. The same or greater effects are expected upon administration of a urethral suppository.

What is claimed is:

1. A method for treating erectile dysfunction in a male individual, comprising administering to the individual a therapeutically effective amount of a pharmaceutical formulation comprised of a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof, wherein the formulation is administered transurethrally, topically, or transdermally.

2. The method of claim 1, wherein the formulation further comprises a pharmaceutically acceptable carrier.

3. The method of claim 1, wherein the Type III phosphodiesterase inhibitor is selected from the group consisting of bipyridines, imidazolones, imidazolines, dihydropyridazinones, dihydroquinolones, mixed Type III-Type IV inhibitors, anagrelide, bemoradan, ibudilast, isomazole, lixazinone, motapizone, olprinone, phtbalazinol, pimobendan, quazinone, siguazodan and trequinsin.

4. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a bipyridine.

5. The method of claim 4, wherein the bipyridine is selected from the group consisting of amrinone and milrinone.

6. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is an imidazolone.

7. The method of claim 6, wherein the imidazolone is selected from the group consisting of piroximone and enoximone.

8. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is an imidazoline.

9. The method of claim 8, wherein the imidazoline is selected from the group consisting of imazodan and 5-methyl imazodan.

10. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a dihydropyridazinone.

11. The method of claim 10, wherein the dihydropyridazinone is selected from the group consisting of indolidan and LY 181512.

12. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a dihydroquinolinone.

13. The method of claim 12, wherein the dihydroquinolinone is selected from the group consisting of cilostamide, cilostazol, vesnarinone and OPC 3911.

14. The method of claim 3, wherein the Type III phosphodiesterase inhibitor is a mixed Type III–Type IV phosphodiesterase inhibitor.

58. The formulation of claim 52, wherein the additional active agent is selected from the group consisting of nitrovasodilators, alpha receptor blocking agents, ergot alkaloids, naturally occurring, semisynthetic and synthetic prostaglandins; vasoactive intestinal peptide; and combinations thereof.

59. The formulation of claim 58, wherein the additional active agent is a prostaglandin.

60. The formulation of claim 59, wherein the additional active agent is PGE_1.

61. The formulation of claim 58, wherein the additional active agent is an alpha receptor blocking agent.

62. The formulation of claim 61, wherein the additional active agent is prazosin.

63. A Pharmaceutical formulation for treating erectile dysfimction, comprising a therapeutically effective amount of a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof, and a pharmaceutically acceptable carrier suitable for administration of a drug to the skin or mucosal tissue.

64. The formulation of claim 63, wherein the formulation comprises an ointment, cream, gel or lotion.

65. A kit for treating erectile dysfunction, comprising: a pharmaceutical formulation comprising a Type III phosphodiesterase inhibitor or a pharmaceutically acceptable salt, ester, amide or prodrug thereof; a means for locally administering the formulation; a container for housing the formulation and drug delivery means; and instructions for using the drug delivery means to administer the formulation within the context of a dosing regimen effective to treat erectile dysfuction.

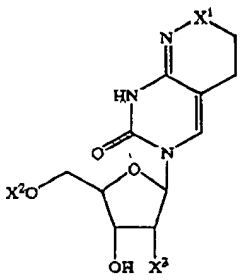
66. The kit of claim 65, wherein the means for administering the agent is a transurethral drug delivery device.

67. The kit of claim 65, further including a flexible, adjustable venous flow control (VFC) device and instructions for using the VFC device.

* * * * *

We claim:

1. A compound having the structure:



where X^1 is selected from the group consisting of O, S, N-alkyl, N^+-dialkyl and N-benzyl; X^2 is selected from the group consisting of triphosphate $(P_3O_9)^{4-}$, diphosphate $(P_2O_6)^{2-}$ and thiotriphosphate $(P_3O_8S)^{4-}$; and X^3 is selected from the group consisting of H, NH_2, F and OR, where R is H, methyl, allyl or alkaryl.

2. A compound according to claim 1, wherein X^1 is O.

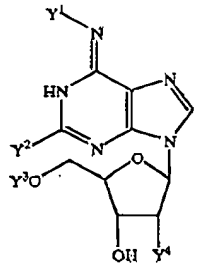
3. A compound according to claim 2, wherein, X^2 is triphosphate, and X^3 is H or OH.

4. A method of mutating a nucleic acid sequence, comprising replicating a template sequence in the presence of a nucleoside triphosphate analogue in accordance with claim 1, so as to form non-identical copies of the template sequence having one or more nucleoside phosphate analogue residues.

5. A method according to claim 4, comprising replicating a template sequence in the presence of deoxyP triphosphate, so as to form non-identical copies of the template sequence having one or more dP nucleotide residues.

6. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of one or more additional nucleoside triphosphates.

7. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of a second compound having the structure:



where Y^1 is selected from the group consisting of OH, O-alkyl, NH_2 and $N(Alkyl)_2$; Y^2 is selected from the group consisting of H and NH_2; Y^3 is selected from the group consisting of triphosphate $(P_3O_9)^{4-}$, diphosphate $(P_2O_6)^{2-}$ and thiotriphosphate $(P_3O_8S)^{4-}$; and Y^4 is selected from the group consisting of H, NH_2, F and OR, where R is H, methyl, allyl or alkaryl.

8. A method according to claim 4, further comprising wherein the template sequence is replicated in the presence of at least one member of the group consisting of 2'-deoxy-8-hydroxyguanosine 5'-triphosphate, 2-amino-9-(2-deoxy-β-D-erythropentofuranosyl)-6-methoxyaminopurine 5'-triphosphate, and O^2-ethylthymidine triphosphate.

9. A method according to claim 4, further comprising the step of replicating the non-identical copies of the template sequence in the presence of the four normal dNTPs, but in the absence of analogues thereof, to form further non-identical copies of the template sequence comprising only the four normal deoxynucleotides.

10. A method according to claim 4, wherein the replication of the template sequence, and/or the replication of the non-identical copies thereof, is achieved by means of PCR.

11. A method according to claim 4, further comprising wherein the template sequence is replicated in the additional presence of the four normal deoxynucleotides.

12. A method according to claim 5, wherein the template sequence is replicated in the presence of 1 μM to 600 μM 6-(2-deoxy-β-D-erythropentofuranosyl)-3,4 dihydro-8H-pyrimido [4,5-c][1,2]oxazine-7-one 5' triphosphate.

13. A method according to claim 5, wherein the template sequence is replicated in the presence of 1 μM to 600 μM 2'-deoxy-8-hydroxyguanosine 5'-triphosphate.

14. A kit for performing the method of claim 4, comprising said nucleoside triphosphate analogue means for replicating a template sequence so as to incorporate the nucleoside monophosphate portion of the nucleoside triphosphate analogue into non-identical copies of the template sequence, and instructions for use according to said method.

15. A kit according to claim 14, wherein the nucleoside triphosphate analogue is 6-(2-deoxy-β-D-erythropentofuranosyl)-3,4 dihydro-8H-pyrimido [4,5-c][1,2]oxazine-7-one 5'triphosphate.

16. A kit according to claim 14, further comprising wherein the means for replicating the template sequence comprises means for performing the polymerase chain reaction.

17. A kit according to claim 14, further comprising the four normal deoxynucleotides.

18. A kit according to claim 14, further comprising at least one member of the group consisting of 2'-deoxy-8-hydroxyguanosine 5'-triphosphate, 2-amino-9-(2-deoxy-β-D-erythropentofuranosyl)-6-methoxyaminopurine 5'-triphosphate, and O^2-ethylthymidine triphosphate.

* * * * *

5. The method of claims 1–4, wherein the MEK1 inhibitor is administered to a subject who has had an ischemic stroke.

6. The method of claims 1–4, wherein the MEK1 inhibitor is administered prophylactically to a subject at risk of having an ischemic stroke.

7. A pharmaceutical composition comprising

a MEK1 inhibitor, and

a non-MEK1 inhibitor anti-stroke agent, together in an amount effective for treating an ischemic condition.

8. A kit comprising a package housing

a first container containing a MEK1 inhibitor, and

instructions for using the MEK1 inhibitor in the treatment of an ischemic condition.

9. The kit of claim 8, further comprising

a second container containing a non-MEK1 inhibitor anti-stroke agent.

10. A medical product comprising

an isolated organ in a perfusion fluid containing a MEK1 inhibitor.

11. A medical product comprising an organ perfusion fluid containing a MEK1 inhibitor.

* * * * *

12. The bioassay according to claim 1, wherein the chemical substance is an herbicide, insecticide, fungicide, xenobiotic, plasticizer, phytohormone, phytoestrogen, organic solvent, or a combination thereof.

13. The bioassay according to claim 12, wherein the chemical substance is selected from the group consisting of estradiol, diethylstilbestrol, kepone, dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane, 1-hydroxychlordene, chlordane, zearalenone, coumestrol, nonylphenol, butylphenol, pentylphenol, isopentylphenol, polychlorinated biphenyl, chlorpyrifols, pentachlorophenol, atrazine, carbaryl, endosulfan, ethanol, and derivatives thereof.

14. The bioassay according to claim 1, wherein the substance is atrazine.

15. A bioassay for determining the sublethal level of toxicity of a substance, comprising:

maintaining a control medium and a series of dilutions of the substance in an aqueous medium under growth conditions to induce sexual reproduction in Daphnia, each of the dilutions and the control containing an effective number of adult, oviparous Daphnia of a single clone for crowding;

determining the sublethal level of toxicity of the substance by comparing fecundity and survivorship of Daphnia in the dilutions and the control to identify the dilution having the highest concentration of the substance at which survivorship and fecundity are at about the same level as the control.

16. The bioassay according to claim 15, further comprising:

comparing one or more endpoints that indicate a deviation from normal Daphnia sexual reproduction in the test sample dilution having the highest sublethal concentration of the substance.

17. The bioassay according to claim 15, wherein the endpoints include the number of male offspring, number of female offspring, sex ratio of males: total offspring, number of resting eggs, and number of offspring displaying a morphological abnormality, number of offspring displaying a behavioral abnormality, and nutritional status of offspring.

18. A bioassay for determining the presence of a toxic chemical substance in a sample, comprising:

maintaining a control medium and the sample in an aqueous medium under growth conditions to induce sexual reproduction in Daphnia, each of the control and the sample containing an effective number of adult, oviparous Daphnia of a single clone for crowding; and

comparing fecundity and survivorship of the Daphnia in the sample and the control;

wherein the presence of a toxic substance in the sample is determined by a lower level of fecundity, and survivorship of the Daphnia in the sample compared to the control.

19. A kit for use in conducting a Daphnia reproductive bioassay on an aqueous sample to detect a substance that interferes with endocrine function in an animal, comprising, in association and separately packaged:

a culture of a clone of Daphnia capable of producing at least about 5–70% males of the total offspring under control conditions; instructions for conducting the Daphnia bioassay according to claim 1; a data scoring sheet; and an algal food source for the Daphnia.

20. The kit according to claim 19, further comprising:

a container for the sample; a container for the control; a container for observing the Daphnia; a device for manipulating the Daphnia; a growth medium for culturing the Daphnia and the algae; or any combination thereof.

* * * * *

What is claimed is:

1. A method of isolating a recombinant adeno-associated virus, comprising applying a sample containing recombinant adeno-associated virus to an iodixanol gradient, and collecting said recombinant adeno-associated virus from said gradient.

2. The method of claim 1, wherein said iodixanol gradient is a discontinuous gradient.

3. The method of claim 2, wherein said iodixanol gradient comprises an about 15% iodixanol step, an about 25% iodixanol step, an about 40% iodixanol step, and an about 60% iodixanol step.

4. The method of claim 3, wherein said recombinant adeno-associated virus is collected from said 40% iodixanol step.

5. The method of claim 3, wherein said 15% iodixanol step further comprises about 1 M NaCl.

6. The method of claim 1, wherein said iodixanol gradient is subjected to centrifugation after applying said sample.

7. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix, removing non-bound species from said matrix, and eluting said virus from said matrix.

8. The method of claim 7, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.

9. The method of claim 7, wherein said matrix is comprised within an HPLC column.

10. The method of claim 7, wherein said virus is eluted from said matrix with a solution comprising about 1 M NaCl.

11. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix, and collecting non-interacting virus from said hydrophobic matrix.

12. The method of claim 11, wherein said hydrophobic matrix comprises phenyl groups.

13. The method of claim 12, wherein said hydrophobic matrix is phenyl-sepharose.

14. The method of claim 1, further comprising applying said recombinant adeno-associated virus to a cesium chloride equilibrium density gradient, and collecting said recombinant adeno-associated virus from said gradient.

15. The method of claim 1, further comprising contacting said recombinant adeno-associated virus with at least a first ion exchange chromatography medium, under conditions effective to permit interaction of said virus with said medium, removing non-interacting species from said medium, and eluting said virus from said medium.

16. The method of claim 1, wherein said sample further comprises a virus.

17. The method of claim 16, wherein said sample further comprises an adenovirus.

18. The method of claim 1, wherein said sample further comprises at least a first polypeptide or protein.

19. The method of claim 1, wherein said sample further comprises a cell extract or a growth medium.

20. A method of isolating a recombinant adeno-associated virus, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix; and

e) eluting said virus from said matrix.

21. A method of isolating a recombinant adeno-associated virus, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix;

e) eluting said virus from said matrix;

f) contacting the eluted virus with a hydrophobic matrix, under conditions effective to permit interaction of hydrophobic species with said hydrophobic matrix; and

g) collecting the non-interacting virus from said hydrophobic matrix.

22. A method for reducing or eliminating adenovirus from a recombinant adeno-associated virus composition contaminated with adenovirus, comprising applying a sample containing recombinant adeno-associated virus and adenovirus to an iodixanol gradient, and collecting from said gradient at least a first fraction comprising said recombinant adeno-associated virus.

23. A method of producing a recombinant adeno-associated virus having a particle-to-infectivity ratio of less than about 100 to 1, comprising the steps of:

a) centrifuging a sample containing recombinant adeno-associated virus through an iodixanol gradient;

b) collecting from said iodixanol gradient at least a first fraction comprising said recombinant adeno-associated virus;

c) contacting said at least a first fraction comprising said recombinant adeno-associated virus with a matrix comprising heparin, under conditions effective to permit binding of said virus to said matrix;

d) removing non-bound species from said matrix; and

e) eluting said virus from said matrix.

24. A kit comprising, in a suitable container, iodixanol, a matrix comprising heparin and instructions for isolating recombinant adeno-associated virus.

25. The kit of claim 24, wherein said iodixanol is formulated as an iodixanol gradient.

26. The kit of claim 24, wherein said matrix comprises heparin agarose type I or heparin agarose type II-S.

27. The kit of claim 24, further comprising a hydrophobic matrix.

28. The kit of claim 27, wherein said hydrophobic matrix comprises phenyl groups.

29. The kit of claim 28, wherein said hydrophobic matrix is phenyl-sepharose.

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We claim:

1. A method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction product that increases visibility of said cells on Pap smears, comprising the steps of:

a) scrapping cervical epithelium by an abrasive device,

b) obtaining an analytical specimen composed of cervical cells from more than one layer of cervical epithelium,

c) spreading said specimen over a microscopic slide to form a smear of separated cells,

d) exposing said smears to air fixation before transporting to a laboratory,

e) exposing said smears to fixation with a solution containing at least citrate acetone or formaldehyde at room temperature,

f) incubating said smears, after fixation, with an incubation mixture containing at least a diazonium salt and a naphthol phosphate in water, for time, temperature and pH, optimal for said acid phosphatase reaction to complete,

g) exposing said smears, after incubation, to staining with a modified Papanicolaou staining method,

h) mounting said smears after completion of said Papanicolaou staining, and

i) investigating said smears, after mounting, under a microscope using new criteria for classification of smears stained by the CAP-PAP test,

whereby said method allow a human observer, or a machine, to diagnose said specimens into categories of negative, nonnegative or positive for cervical dysplasia and/or cervical cancer.

2. A kit which comprises a carrying box comprising:

a) labeled bottles containing reagents for use in a method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction,

b) control slides, comprising
(1) microscopic slides with HeLa cells smeared on, and
(2) microscopic slides stained with HeLa cells, and

c) written instructions for using said reagents to perform said method on analytical specimens obtained from healthy women or patients, and

d) written instructions for using said HeLa smears for quality control and/or quality assurance of said method.

3. An assembly of instruments comprising at least

a) an automatic mechanical device to combine all steps of a method for in vitro labeling of abnormal cervical cells with an acid phosphatase enzyme reaction into a continuous stepwise procedure, comprising:
i) a train to carry unstained smears throughout a series of stations, and
ii) a series of stations providing technical facility for performance of every step of the staining procedure, and
iii) a motor to move said train with smears throughout said stations and said staining procedure,
whereby said devices are combined into an automatic staining device for processing said marker and mak-